

## Investigation on the Surface Hydrophobicity and Aggregation Kinetics of Human Calprotectin in the Presence of Calcium

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Calcium and zinc binding protein, calprotectin is a multifunctional protein with broad spectrum antimicrobial and antitumoural activity. It was purified from human neutrophil, using a two-step ion exchange chromatography. Since surface hydrophobicity of calprotectin may be important in membrane anchoring, membrane penetration, subunits oligomerization and some biological roles of protein, in this study attempted to explore the effect of calcium in physiological range on the calprotectin lipophilicity. Incubation of human calprotectin (50 µg/ml) with different calcium concentrations showed that 1-anilino-8-naphthalene sulfonic acid (ANS) fluorescence intensity of the protein significantly elevates with calcium in a dose dependent manner, suggesting an increase in calprotectin surface hydrophobicity upon calcium binding. Our study also indicates that calcium at higher concentrations (6, 8 and 10 mM) induces aggregation of human calprotectin. Our finding demonstrates that the starting time and the rate constant of calprotectin aggregation depend on the calcium concentration.

**Keywords:** Aggregation, ANS-binding fluorescence, Calcium, Calprotectin, Hydrophobicity, Neutrophil

### Introduction

Myeloid-related protein (MRP) 8 and MRP14 are two small anionic proteins, with zinc and calcium binding capacity; they belong to S-100 protein family and abundantly found in cytosolic fraction of neutrophils (Hessian *et al.*, 1993; Bella and Rossmann, 1999). They form a heterodimeric complex in a calcium dependent manner that called MRP8/14,

calprotectin or cystic fibrosis antigen (Steinbakk *et al.*, 1990; Murao *et al.*, 1998). Human MRP8 and MRP14 have molecular masses of 11 and 14 kDa with 93 and 114 amino acids, respectively. Each subunit of the protein is composed of two distinct calcium binding motifs (EF-hand) flanked by hydrophobic regions at either terminus and separated by a central hinge region (Ordink *et al.*, 1987; Hessian *et al.*, 1993).

Calprotectin shares properties with calmodulin but differ in molecular mass and it would be expected to exert a major biological effect by modifying the intracellular calcium level (Burgess *et al.*, 1980). High concentration of calprotectin in neutrophil acts as calcium sink and may be protecting cells from harmful effect of prolonged calcium elevation.

Calcium is a key secondary messenger during signal transduction and calprotectin may binds to free intracellular calcium and interfere with signal transduction (Heizmann and Hunziker, 1991).

Calprotectin is a multifunctional protein with broad spectrum antimicrobial and antitumoural effects (Steinbakk *et al.*, 1990; Satoru *et al.*, 1997) that is significantly elevated in the serum and body fluids of patients with cystic fibrosis (Dorin *et al.*, 1987) and inflammatory states such as rheumatoid arthritis (Berntzen *et al.*, 1991), Crohn's disease (Lawrance *et al.*, 2001) colorectal carcinoma (Tibble *et al.*, 2001), multiple sclerosis (Bogumil *et al.*, 1998) and human immunodeficiency virus (HIV) infection (Müller *et al.*, 1994). Also the large subunit of calprotectin is expressed in brain tissue of those suffering from the amyloid disease, Alzheimer (Akiyama *et al.*, 1994).

A variety of possible functions have been proposed for calprotectin such as the capacity for binding to poly unsaturated fatty acids (PUFAs) governed through calcium binding (Klempt *et al.*, 1997; Kerkhoff *et al.*, 1999). Investigators have shown that calprotectin inhibits the activity of casein kinases I and II (Murao *et al.*, 1989), two enzymes that mediate RNA polymerase (Stetler *et al.*, 1982; Rose *et al.*, 1983) and topoisomerase activity (Ackerman *et al.*, 1985).

Research also reveals that calcium binding induces

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